

PUTATIVE HYBRIDIZATION IN THE GENUS EUPATORIUM (COMPOSITAE)¹

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Fryar (1964) and Maurushat (1969) gave evidence for the hybrid origin of several anomalous biotypes in the genus *Eupatorium*. Hybridization in the genus has also been reported by others (Radford, et. al., 1968; Gleason & Cronquist, 1963; Gleason, 1952). This paper concerns the putative hybrid origin of *E. fernaldii* Godfrey from *E. sessilifolium* L. and *E. album* L., the hybridization of *E. sessilifolium* with *E. perfoliatum* Brit., the hybridization of *E. semi-serratum* DC. and *E. lecheaefolium* Greene, and a possible hybrid origin for *E. saltuense* Fern.

MATERIALS AND METHODS

Flowering plants were collected throughout the summer and early fall. Rootstocks to be used as source materials for determining chromosome numbers and investigating breeding systems were taken from the field and replanted in a greenhouse or garden at Florida State University. Herbarium specimens were made of the flowering stems from the field collected plants.

Chromosome analysis was made from the root tips of mature plants, seedlings, or from the pollen mother cells (PMC's) at meiosis. All material was fixed in acetic alcohol, stained with acetorcein, and prepared by the squash technique for observation. Root tips were hydrolyzed in 10% 1N HCl for three to five minutes before staining and squashing. Mitotic material was treated with 0.9% colchicine for an hour prior to fixation. Voucher specimens for chromosome counts (Appendix) were deposited in the FSU Herbarium.

RESULTS AND DISCUSSION

***Eupatorium fernaldii* Godfrey (putative *E. album* × *E. sessilifolium*).**

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Plants of *Eupatorium fernaldii* were studied from twenty-four locations in Georgia and North Carolina. The habitats were gravelly clay and rocky cliff faces adjacent to roads, and open hilly pastures adjacent to pinewoods and mixed hardwood forests in the Montane and Piedmont provinces. Large populations of the biotype occur.

Chromosome counts were made of plants of seven of the populations. Six populations were tetraploid and one was triploid (Appendix). Those plants for which chromosome counts were made and those of the remaining seventeen populations studied failed to produce pollen. A few plants produced grossly malformed pollen without proper wall formation. In order to determine achene setting ability, plants from the seven populations were isolated from pollen sources in a greenhouse; they produced full heads of viable achenes indicating reproduction by agamospermy. Among many of the plants, achene development was observed to occur before anthesis. These are characteristics of polyploid plants of other *Eupatorium* species (Sullivan, 1976).

Plants of *Eupatorium fernaldii* resemble *E. album* L. in having petaloid phyllaries, leaves of similar shape, and a similar habit. However, unlike *E. album*, the leaves are nearly glabrous. Because of the similarity between *E. album* and *E. fernaldii*, Fernald (1937), who originally described this biotype, named it *E. album* var. *monardifolium* Fern. Later, Godfrey (1950) elevated it to the species level. Godfrey (pers. comm.) recently examined an isotype of *E. vaseyi* Porter and found it to be phenotypically like the type of *E. fernaldii*. Previously, Fernald (1945) had reassigned *E. vaseyi* Porter to *E. sessilifolium* var. *vaseyi* (Porter) Fern. and Grisc.

Since diploids of this taxon were not found and due to its similarity to *Eupatorium album*, and its occurrence as male-sterile, polyploid agamics, the hypothesis of a hybrid origin seemed reasonable. Based on the morphological similarity previously mentioned, one obvious hypothetical parent is *E. album*. The other hypothetical parent should occur within the prescribed area of distribution of *E. fernaldii*, as does *E. album*, as a sexual diploid, and have nearly glabrous leaves as does *E. fernaldii*. The only *Eupatorium* with these characteristics is *E. sessilifolium* L. (Sullivan, 1976).

Measurements were made of the three entities, putative parents and hybrid (Table 1). In addition to similarities between *Eupatorium fernaldii* and the putative parents mentioned above, shape and

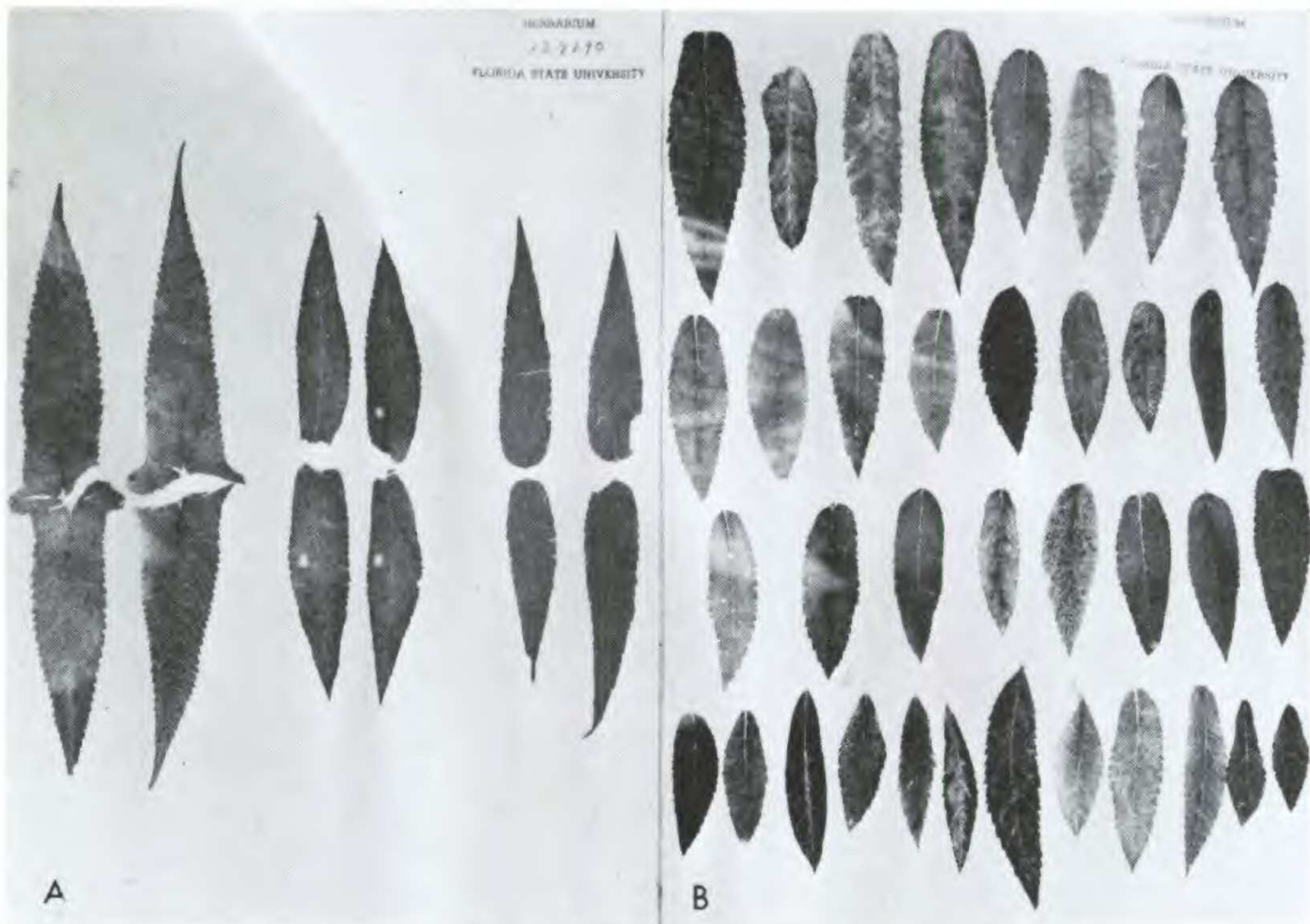


Figure 1A and B. A. (left to right): Two pairs of leaves each of *Eupatorium perfoliatum*, hybrid, and *E. sessilifolium*. B. Leaves from backcross hybrid plants, *Eupatorium sessilifolium* × hybrid.

serration of leaves of *E. fernaldii* plants were intermediate between *E. album* and *E. sessilifolium*; leaf length tended to be like *E. album*, while leaf width exceeded that of both the parental plants. The lengths of capitulary characters of hybrid plants tended to equal or to exceed those of *E. album* plants.

Although further evidence is needed, the morphological evidence provided suggests a hybrid origin for *Eupatorium fernaldii*. Evidence from phytochemical and karyotypic work might provide further information not readily apparent from the morphology.

***Eupatorium sessilifolium* × *perfoliatum*.**

The distributions of *Eupatorium sessilifolium* (discussed above) and *E. perfoliatum* overlap throughout the range of the former. *Eupatorium perfoliatum* has a broad distribution throughout eastern North America and parts of the midwest. While collecting in the southern Appalachians in Graham County, North Carolina, I found one plant intermediate in morphology between *E. sessilifolium* and

Table 1. Morphological comparison of *Eupatorium album*, *E. sessilifolium* and *E. fernaldii*.

	<i>E. album</i> var. <i>album</i>	<i>E. fernaldii</i>	<i>E. sessilifolium</i>
Leaves:	4.8–11 cm long, 1–3.4 cm wide, elliptic with acute tips and attenuate bases, 10–30 teeth; crenate and double crenate, irregularly spaced, nearly cleft in some upper leaves, short petiolate, densely pilose on upper and lower surfaces	4.8–11 cm long, 2–3.8 cm wide, elliptic to lanceolate, acute to acuminate tips, bases sessile, 5–23 teeth doubly serrate, scattered pilose along veins of upper and lower surfaces	6–10 cm long, 1.6–3.2 cm wide, lanceolate with acuminate tips, clasping to rounded bases, sessile, 8–20 teeth, serrate; pilose on upper veins, lower surfaces glabrous except mid veins
Head:	7–10 mm	7–9 mm	6–7 mm
Corolla:	4–5 mm	4–5.5 mm	2.5–3 mm
Pappus:	4–5.5 mm	4–6 mm	3.5–4 mm
Phyllaries:	10–15, white petaloid, little green, 7–10 mm, acuminate	10–14 white petaloid margins and tip, 7–9 mm mucronate-caudate	9–12 green with hyaline margin, 3.5–5 mm, acute

E. perfoliatum; the intermediate plant was growing within a large population of diploid *E. sessilifolium* plants along a disturbed and eroded bank. *Eupatorium perfoliatum* was nearby in a poorly drained area on a road bank.

The diploid intermediate was transplanted to a greenhouse where reciprocal backcross hand-pollinations were made to diploid *Eupatorium sessilifolium*. The achenes were sown, and the resulting progeny were like those of *E. sessilifolium*; however, a few had clasping to perfoliate bases, like the *E. perfoliatum* and intermediate plant (Figures 1A and B), indicating hybridity.

Meiosis in most PMC's of the hybrid was normal; however, in 7.3% of the cells observed two univalents were formed (Table 2). In 2.5% of the cells at Telophase II two micronuclei were found; those probably resulted from failure of laggards to be included in the large nuclei.

Despite the paucity of hybrid collections of this combination, hybridization could occur more frequently than observed since the species are sympatric in a part of their range. However, ecological barriers might prevent establishment. The high degree of interfertility, as indicated by chromosomal compatibility, and the capacity to form backcross progeny with at least the one parent tested indicate that genetic isolating barriers are poorly defined. Further examination of the two species could reveal that introgression is occurring, if sufficiently strong ecological barriers to hybrid establishment do not exist.

***Eupatorium semiserratum* × *lecheaefolium*.**

Eupatorium semiserratum plants occur as sexual diploids throughout their distributional range; the distribution is Coastal Plain and adjacent areas from Florida to eastern Texas, north to Virginia, Tennessee, and southeastern Missouri (Sullivan, 1976). The habitats include moist to boggy, peaty, sandy soils in pine flatwoods, margins of seasonal ponds, gum swamps, and bayheads. In addition the plants are abundant in disturbed habitats such as firelanes, margins of roadside ditches, and clear-cut pine flatwoods. Plants of *E. lecheaefolium* Greene occur as diploids ($n = 10$) and triploids (Sullivan, 1976). The triploids were male-sterile and agamosperous; the diploids are sexual, male-fertile and self-incompatible (Sullivan, 1976). Diploid plants of *E. lecheaefolium* are narrowly

Table 2. Meiosis in the field hybrid *Eupatorium perfoliatum* × *sessilifolium*.

No. MI cells	% MI with 2 univalents	No. AI cells	%AI with laggards:		No. TII cells	%TII with micronuclei	
			1	2		1	2
27	7.3	20	0	5	39	0	2.5

distributed in southern Georgia and midnorthern Florida. Grant (1953) made chromosome counts of a diploid from Alexander County, North Carolina; however, field work in that area during this study failed to reveal diploid plants of *E. lecheaefolium*. Herum specimens which were identified as polyploids by their male-sterility were collected in the Coastal Plain and Piedmont from North Carolina to southern New Jersey, and from Georgia to southern Louisiana and to eastern Tennessee (Sullivan, 1976). Plants of this species occur in dry, upland pinewoods and edges of rich woods. Disturbed areas such as firelanes, highway rights-of-way, and old fields also provide favorable habitats.

Diploids of the two species are found intermixed occasionally in disturbed sites where their habitats are contiguous. From one such location (Leon County, Florida) where intermediate plants were present, seeds were collected from putative parental and hybrid plants and progeny tests were made in a greenhouse.

With few exceptions, progeny from the parental plants were like the respective species, indicating that backcrosses are infrequent or unsuccessful. However, progeny from hybrid plants were morphologically diverse, recombining characteristics of the parental species (Figure 2). Figure 3 is a scatter diagram made by using leaf width and number of teeth per one leaf margin of the progeny. The figure illustrates the intermediacy and recombinance of parental characters in the hybrids.

A year later over half the greenhouse progeny had died. The remaining plants were remeasured and a second scatter diagram constructed (Figure 4) again using leaf width and number of teeth per margin. The surviving progeny, as illustrated in the scatter diagram, were the plants resembling the parents; the intermediate plants had died. These results suggest that the intermediate condition is intrinsically unstable. No competition occurred in the greenhouse pots and no other apparent extrinsic selection occurred.

F₁ hybrid plants were synthesized by reciprocal hand pollination between diploids of *Eupatorium semiserratum* and *E. lecheaefolium*. A total of six hybrids was obtained from the *E. semiserratum* parent and none from the *E. lecheaefolium* parent. Self-pollinations, intersibling crosses, and reciprocal backcrosses to *E. semiserratum* were made using the F₁ hybrids. Self-pollinations and intersibling crosses failed to produce viable seeds; however backcrosses

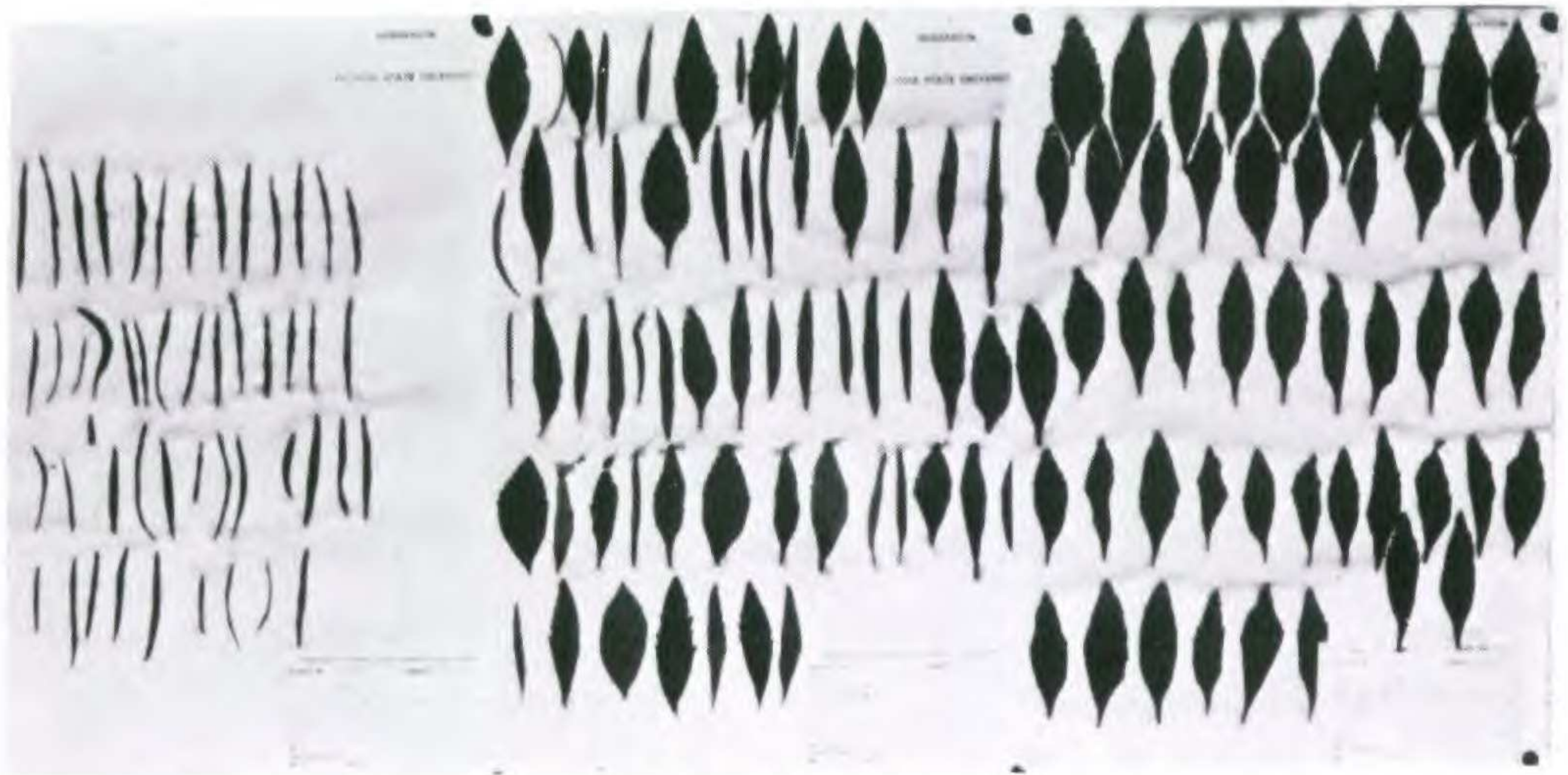


Figure 2. Leaves from sixth node of plants from progeny test. Left, leaves from progeny of *Eupatorium lecheaefolium*; center, leaves from progeny of intermediate plants; right, leaves from progeny of *E. semiserratum*.

yielded thirteen progeny from the *E. semiserratum* seed parent and seven progeny from the F_1 seed parent.

Analyses of meiosis were made of three F_1 hybrids (Table 3). Meiosis was irregular with a high frequency of two and four univalents, one or two nondisjunct bivalents, and lagging univalents at Anaphase I. In one hybrid, one to three chromatids were excluded from the Metaphase II plate.

The differences in habitats of the parental species, intrinsic selection against intermediate hybrids, apparent infrequency of backcrossing in the field and meiotic irregularities are factors which isolate the species.

Some of the hybrid progeny resembled plants referred to as *Eupatorium hyssopifolium* L. var. *laciniatum* Gray and var. *hyssopifolium*. Numerous collections from populations of these varieties were made for greenhouse culture and study. From those plants, chromosome numbers were determined to be triploid and tetraploid (Appendix). The plants were male-sterile; however, when isolated in a greenhouse from pollen sources, they produced full heads of viable achenes. Progeny grown from these achenes had the same chromosome numbers as the respective parent plants. Those results reveal the plants to be agamic, as has been discovered in other *Eupatorium* polyploids (Sullivan, 1976). Grant (1953) gave triploid chromosome counts for *E. hyssopifolium* also.

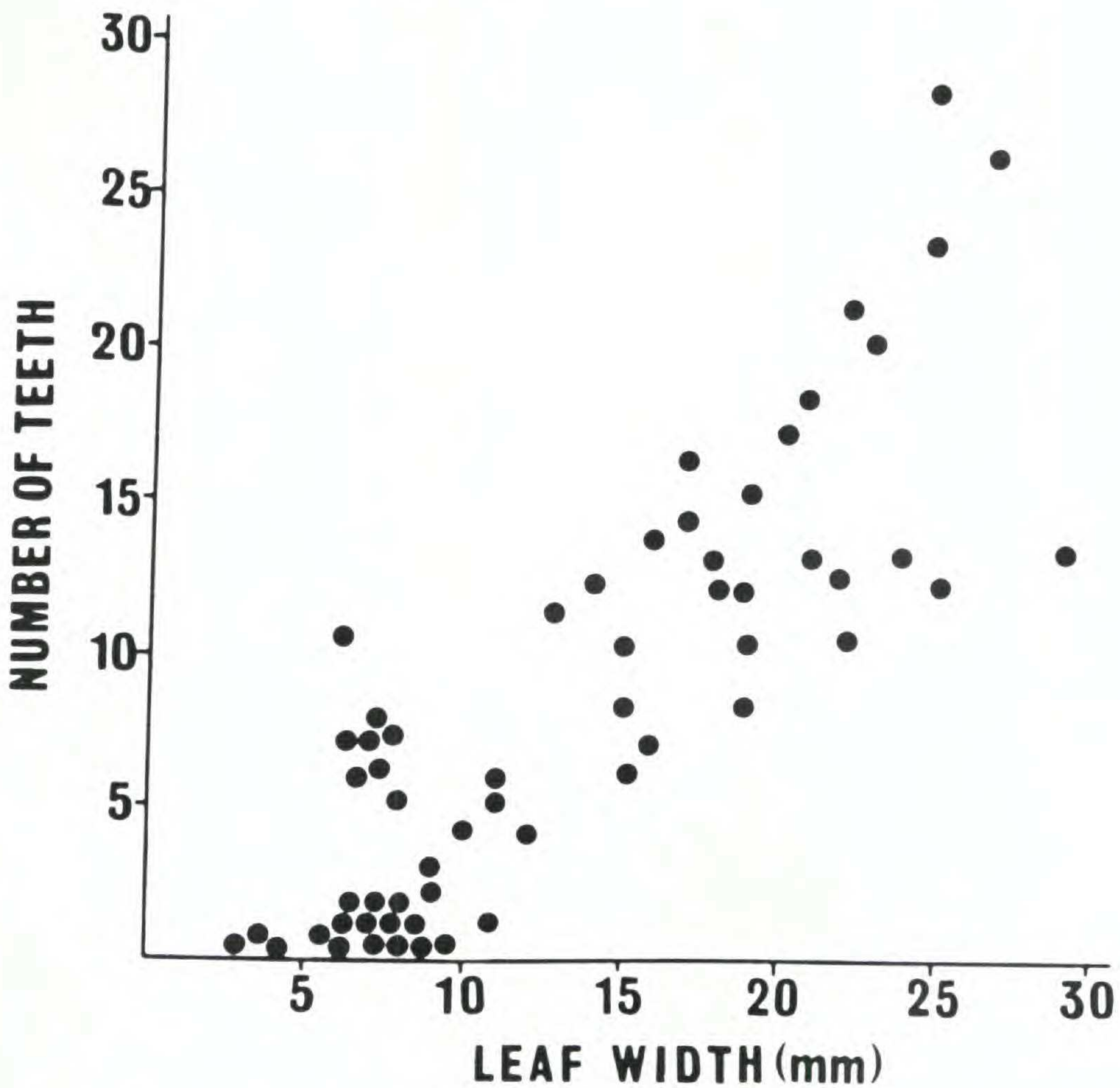


Figure 3. Scatter diagram of progeny from test of naturally occurring hybrids of *Eupatorium semiserratum* × *lecheaefolium* and parental plants.

Plants of those varieties are variable. Fernald (1950) suggested that the two mentioned above and *Eupatorium hyssopifolium* L. var. *calcaratum* Fern. and Schub., which is synonymous with *E. lecheaefolium*, are confluent. The relationship of plants of those varieties to hybrids of *E. lecheaefolium* × *semiserratum* are being investigated through karyotyping and phytochemical studies.

Eupatorium saltuense (putative *E. album* × *E. lecheaefolium*).

Eupatorium saltuense was described by Fernald (1942) from southeastern Virginia. Radford et al. (1968) reported the species from Caswell, Granville, and Warren counties in the North Carolina Piedmont.

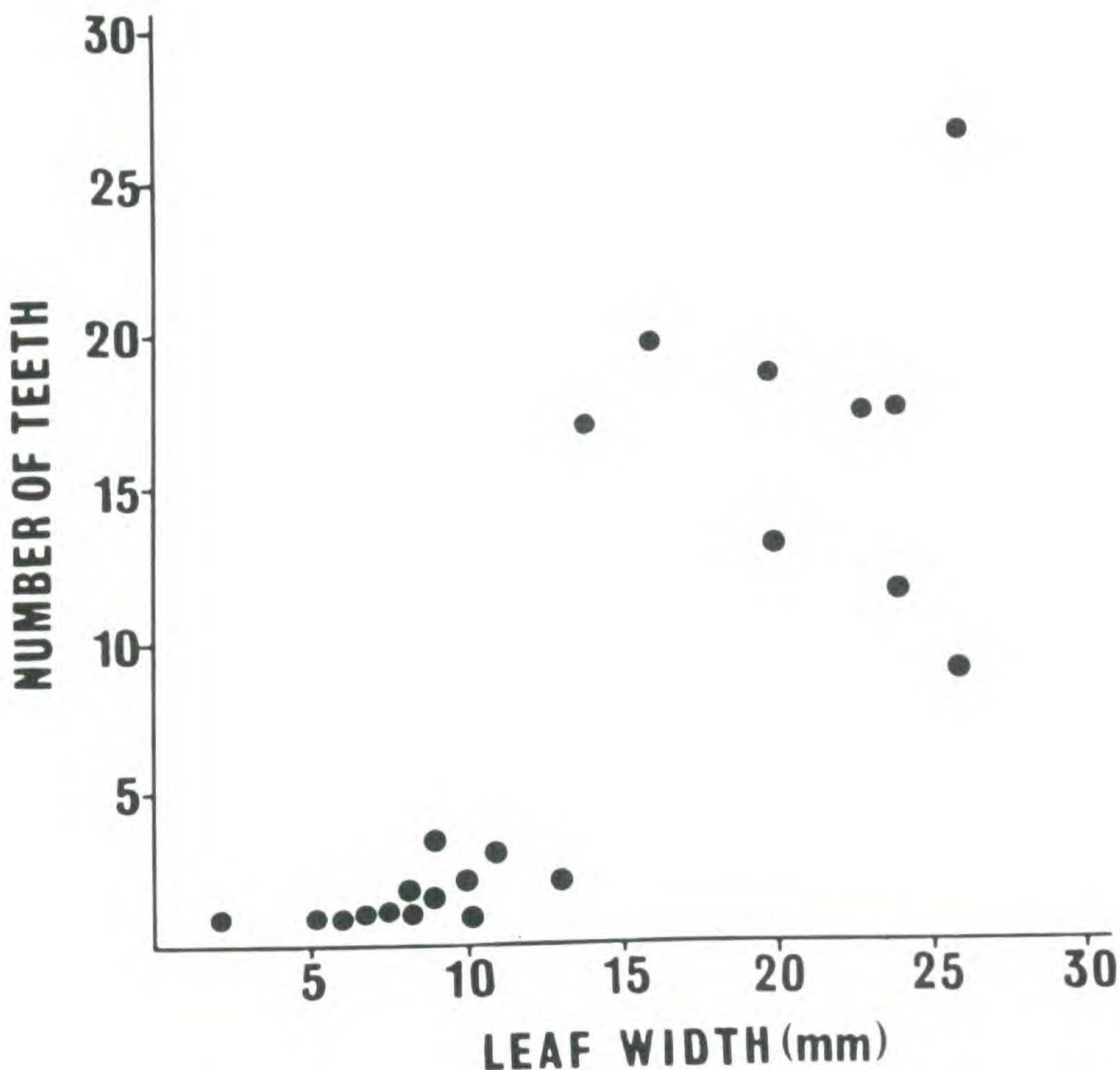


Figure 4. Scatter diagram of same progeny as measured in Figure 2 a year later (after most had died).

Population samples of *Eupatorium saltuense* were obtained from various sites in southeastern Virginia. These were grown in a greenhouse. Chromosome numbers of *E. saltuense* were found to be $2n = 30$, a triploid count based on $x = 10$ (Sullivan, 1976; Grant, 1953). The plants were male-sterile and when isolated from pollen sources in a greenhouse, they set full heads of viable achenes revealing their agamic reproductive ability. The male-sterile agamic nature of polyploid eupatoria has been repeatedly found by the author (1976).

The plants were found in disturbed habitats intermixed with polyploid *Eupatorium altissimum* L., triploid *E. lecheaefolium* Greene and two other anomalous polyploids. Diploid plants of *E. album* L. were in the near vicinity.

Table 3. Meiosis in diploid F₁ hybrids of *Eupatorium lecheaefolium* × *semiserratum*.

Plant	No. MI cells	% MI with		No. AI cells	% AI with laggards:						No. MII cells	% MII with			No. TII cells	% TII with micronuclei			
		9II, 2I	8II, 4I		I		II					I II	Excluded I			1	2	3	
					1	2	1	2	3	4			1	2					3
A	28	39	35	1	-	-	-	-	-	-	-	-	-	-	61	16	13	0	
B	-	-	-	33	3	3	9	6	0	0	-	-	-	-	-	-	-	-	
C	8	62	0	82	9	1.2	11	12	1.2	1.2	36	5.4	11	3	3	121	2.4	12	0.8

¹None examined.

Table 4. Morphological descriptions of *Eupatorium album* (2*n*), *E. lecheaefolium* (2*n*), and *E. saltuense*.

	<i>E. album</i>	<i>E. lecheaefolium</i>	<i>E. saltuense</i>
Leaves:	4.8–11 cm long, 1–3.4 cm wide, elliptic with acute tips and attenuate bases, 10–30 teeth; crenate and double crenate, irregularly spaced, nearly cleft in some upper leaves, short petiolate, densely pilose on upper and lower surfaces	2–4 cm long, 0.2 cm wide, linear with acute tip and cuneate base, lower surfaces hispid, upper nearly glabrous, margins entire	4–11.2 cm long, 0.6–3.6 cm wide, elliptic to elliptic-lanceolate, attenuate-acuminate tip, and acute base, glabrous above, pubescent beneath, 2–25 coarse teeth per margin
Phyllaries:	10–15, white petaloid, little green, 7–10 mm, acuminate	mucronate, 3.2–4 mm long	mucronate, 4–6 mm long
Head:	7–10 mm	4.3–5.5 mm long	5–8 mm long
Corolla:	4–5 mm	2.5–3.5 mm long	3–5 mm long
Pappus:	4–5.5 mm	3.2–4 mm long	4–5 mm long

Plants of *Eupatorium saltuense* resemble *E. lecheaefolium* in several respects: suppressed axillary shoots, similar inflorescences, and three prominent veins from the leaf base (Table 4). On the other hand, the leaf is elliptic-lanceolate and its margins are coarsely dentate, unlike the narrow linear one of *E. lecheaefolium* but similar to that of *E. album*.

For the reason stated above, measurements were made of population samples of diploid *Eupatorium lecheaefolium*, *E. album*, and *E. saltuense*. Type and isotype specimens from the Gray Herbarium were also measured and these data are included in the scatter diagram (Figure 5) using involucre bract length and teeth/margin. In the diagram, the type and isotype measurements fall directly

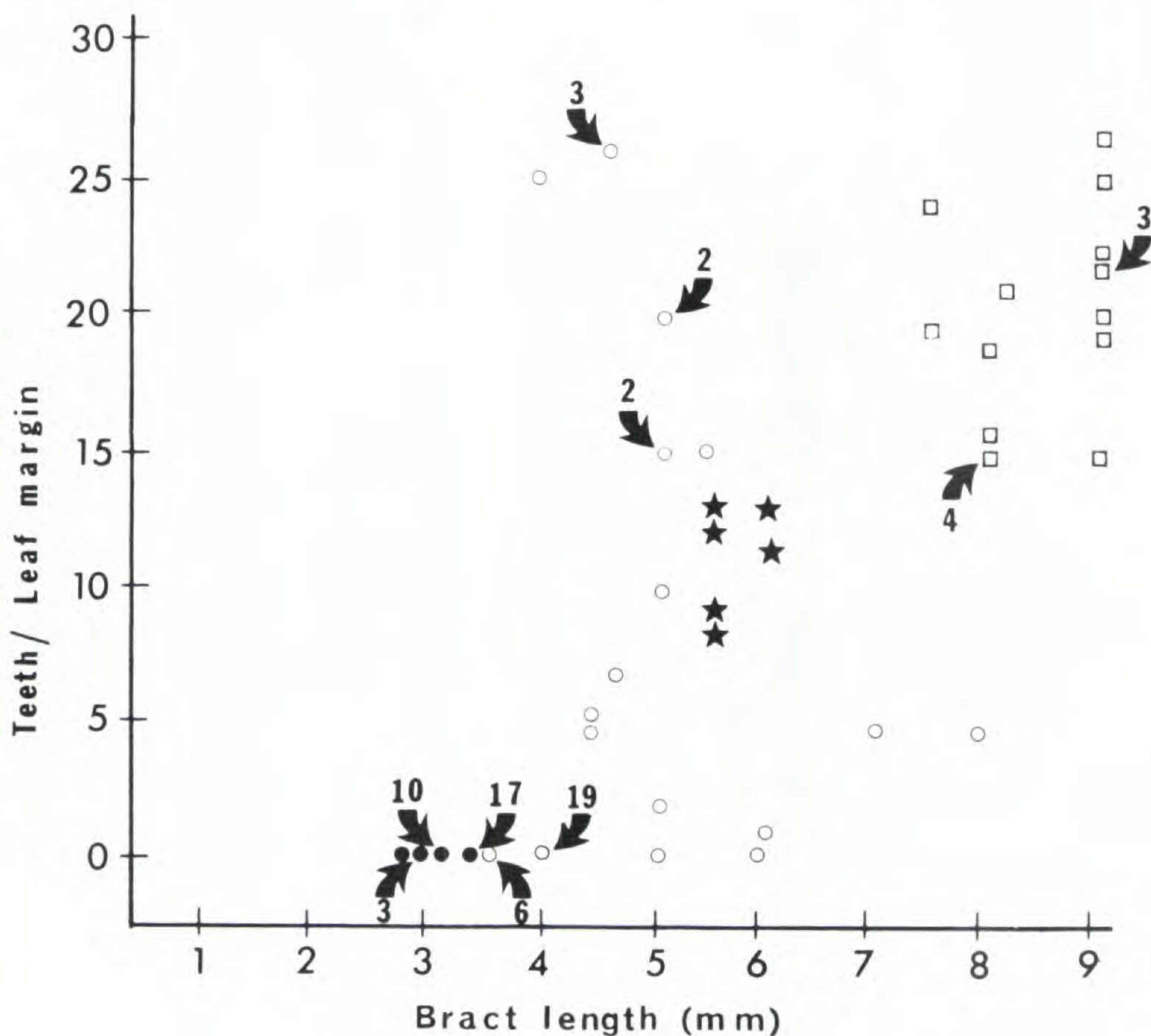


Figure 5. Scatter diagram using measurements of diploid *Eupatorium lecheaefolium* (●) and diploid *E. album* (□), Fernald's types and isotypes of *E. saltuense* (★), and other collections of *E. saltuense* (○). The numbers refer to the specimens with similar measurements represented by the symbol indicated by arrows.

intermediate between the putative parents. Most of the plants collected during this study also fell intermediate; however, some plants exceeded *E. album* in number of teeth per margin.

The origin of *Eupatorium saltuense* from *E. album* \times *lecheaefolium* is uncertain because of the distribution of diploids of the putative parents. Field work during this study revealed that diploids of *E. lecheaefolium* are narrowly distributed in northern Florida and southern Georgia (Sullivan, 1976); Grant (1953) determined diploidy of a specimen from Rocky Face Mountain in the Piedmont Province in Alexander County, North Carolina. A vain search was made for diploids in that vicinity during this study. (By examining pollen, it is a relatively simple matter to determine diploidy of *Eupatorium* specimens.) Because of the apparent dearth of diploid *E. lecheaefolium* in that area it is probable that the origin of *E. saltuense* through hybridization could have occurred in the ancient past when diploids of *E. lecheaefolium* were more prevalent.

Further evidence regarding the origin of *E. saltuense* is being sought from phytochemical and karyotypic studies.

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APPENDIX

***Eupatorium sessilifolium* × *album*: 4n, 3786 R. L. Lazor & V. I. Sullivan, Rabun Co., Ga.; 4n, 3809 RLL & VIS, Jackson Co., N. C.; 4n, 3807 RLL & VIS, Jackson Co., N. C.; 4n, 3810 RLL & VIS, Jackson Co., N. C.; 4n, 3816 RLL & VIS, Jackson Co., N. C.; 4n, 67873 R. K. Godfrey, Jackson Co., N. C.; 3n, 68270 RKG, Buncombe Co., N. C.**

***Eupatorium sessilifolium* × *perfoliatum*: 2n, 3795A RLL & VIS, Graham Co., N. C.**

***Eupatorium hyssopifolium* var. *hyssopifolium* & var. *laciniatum*: 4n, 69777 RKG, Coffee Co., Tenn.; 4n, 69659 RKG, Monroe Co., Ala.; 4n, 68098 RKG, Lamar Co., Miss.; 4n, 69706 RKG, Tuscaloosa Co., Ala.; 3n, 69854 RKG, Montgomery Co., Ala.**

***Eupatorium lecheaefolium* × *semiserratum*: 2n, 68430 RKG, Leon Co., Fla.; 2n, 69860 RKG, Houston Co., Ala.**